Heparin Cell Coating Maintains Cell Number During Mesenchymal Stem Cell Aggregate Culture

Jennifer Lei[1], Johnna S. Temenoff[2]
[1]School of Mechanical Engineering, Georgia Tech; [2]Department of Biomedical Engineering, Georgia Tech/Emory University

Statement of Purpose: Mesenchymal stem cells (MSCs) have been used as cell therapies to aid in regeneration of a variety of injured tissue due to their ability to secrete trophic factors and differentiate down multiple lineages. While MSCs can be formed into 3D aggregates, it has been seen that aggregates decrease almost 50% in size over the course of 21 day in culture[1]. This compaction may be an indication or cytoskeleton and extracellular matrix rearrangement or of cell loss, which can lead to reduction in the aggregates' capacity to promote regeneration. We have previously developed a thin film coating for MSC spheroids using multilayer deposition of biotin and avidin to graft heparin onto cell surfaces[2]. The negative charge of heparin can be decreased by desulfation of the molecule, thus potentially modulating its interactions with soluble factors produced locally by aggregated cells. In order to begin to understand how coating sulfation levels affect communication within cell aggregates, the objective of this study was to determine the effects of heparin and desulfated heparin coatings on MSC aggregate cell morphology and DNA content in media without added serum or growth factors.

Methods: Heparin and desulfated heparin were biotinylated via HOBT/EDC chemistry. Human MSCs were coated as single cells in suspension with sulfo-NHS-biotin (4mM), avidin (0.5mg/mL) and heparin (Hep) or desulfated heparin (Hep-) (5mg/mL). Each layer was incubated for 30 minutes. Once coated, 2000- and 5000-cell aggregates were formed by forced aggregation in Pluronic coated 96-well V-bottom plates and cultured in serum-free MSC maintenance media with media changes every 3 days. Noncoated controls were cultured in serum free maintenance media either with soluble heparin or desulfated heparin (1mg/mL) or neither. On days of sample collection, aggregates were fixed in 10% formalin and processed for sectioning. Samples were sectioned at 10µm and stained with hematoxylin and eosin or with an antibody to α-smooth muscle actin (α-SMA). Additionally, samples were collected for DNA quantification via the CyQUANT assay (n=6). Viscoelastic creep testing using parallel plate compression with a micron-scale mechanical testing system provided deflection data for individual aggregates (Microsquisher; n=3-5). Statistical significance was determined by a two-way ANOVA with Tukey's post hoc test ($p<0.05$).

Results: We have previously shown that fluorescently tagged heparin was observed on cell surfaces for at least 14 days in culture using layer-by-layer deposition[2]. In this study, histological staining revealed that heparin- and desulfated heparin-coated MSCs contained distinctive, rounded cells within large aggregates that were not observed in noncoated MSCs (Fig 1A, white arrows). Immunohistochemical staining demonstrated that there appeared to be less α-SMA found around the border of the heparin- and desulfated heparin-coated aggregates when compared to noncoated samples (Fig 1B). DNA quantification revealed heparin coated MSC aggregate cell number did not decrease significantly over time for both 2000-cell (data not shown) and 5000-cell aggregates (Fig 1C), as compared to noncoated both with and without soluble heparin in the media. The moduli for noncoated, heparin- and desulfated heparin-coated 2000-cell aggregates were not significantly different at any time point (data not shown).

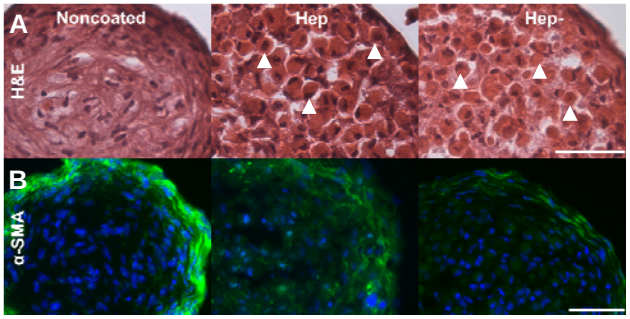

Figure 1. A) Hematoxylin and eosin staining of noncoated, heparin- and desulfated heparin-coated aggregates at d14. Scale bar=50µm. B) α-SMA staining for noncoated, heparin- and desulfated heparin-coated samples at d14. Scale bar=50µm. C) DNA quantification of aggregates. * indicates significant difference from noncoated samples on the same day; $p<0.05$, n=6.

Conclusions: We have observed that while both types of heparin coatings cause morphological differences in MSC aggregates, only the sulfated heparin coating permitted retention in cell number over time in culture. While more mechanistic study is needed, since heparin in soluble form did not have similar effects, the coating presentation appears to be a key component of cell number maintenance. Overall, coated aggregates demonstrated less distinct α-SMA staining than noncoated samples, and thus may not contain as dense of a peripheral boundary, allowing for more diffusion within the aggregate. However, since only heparin-coated samples maintained DNA amount over time, this suggests that there may be interactions beyond simply removing diffusion limitations (such as interactions between the sulfated heparin and any cell-derived growth factors) that drive cell maintenance in aggregates. Such maintenance of cell number in the absence of growth cues from the media can provide a substantial improvement over other pellet-based culture systems and indicates that sulfated heparin-based coating technologies may be used in 3D aggregates to prevent cell loss in scale-up processes for MSC-based therapies.

References: [1]Sart S. Tissue Engineering B. 2014 20(5). [2] Lei J. Biomaterials Science 2014 2: 666.